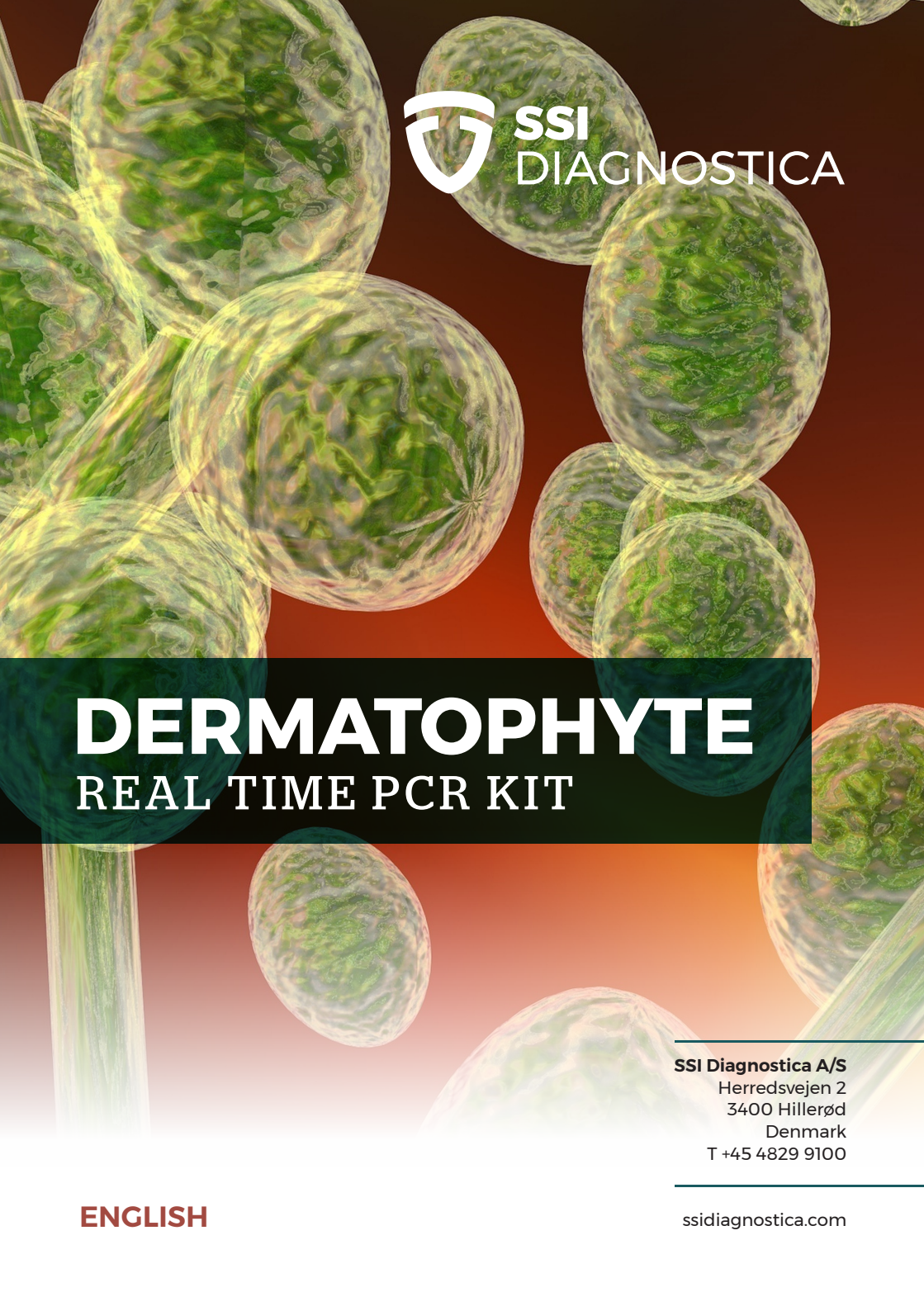

SSI DIAGNOSTICA

DERMATOPHYTE
REAL TIME PCR KIT

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ABBREVIATION LIST

A. flavus	*Aspergillus. flavus*
A. fumigatus	*Aspergillus fumigatus*
A. niger	*Aspergillus niger*
A. wentii	*Aspergillus. wentii*
C. albicans	*Candida albicans*
C. glabrato	*Candida glabrato*
C. krusei	*Candida krusei*
C. parapsilosis	*Candida parapsilosis*
C. tropicalis	*Candida tropicalis*
E. floccosum var. Floccosum	*Epidermophyton floccosum var. Floccosum*
F. oxysporum	*Fusarium oxysporum*
F. proliferatum	*Fusarium proliferatum*
F. solani	*Fusarium solani*
M. audouinii	*Microsporum audouinii*
M. canis	*Microsporum canis*
M. gypseum	*Microsporum gypseum*
S. brevicaulis	*Scopulariopsis brevicaulis*
T. erinacei	*Trichophyton erinacei*
T. interdigitale	*Trichophyton interdigitale*
T. mentagrophytes	*Trichophyton mentagrophytes*
T. rubrum	*Trichophyton rubrum*
T. schoeuleinii	*Trichophyton schoeuleinii*
T. soudanense	*Trichophyton soudanese*
T. tonsurans	*Trichophyton tonsurans*
T. verrucosum	*Trichophyton verrucosum*
T. violaceum	*Trichophyton violaceum*

DERMATOPHYTE REAL TIME PCR KIT

Intended use

The Dermatophyte Real Time PCR Kit is intended for *in vitro* diagnostic detection of dermatophytes in general (pan-dermatophytes) and specifically *Trichophyton (T.) rubrum* in nail specimens.

Description

The kit contains the PCR reagents: Primer and Probe Mix (includes three Primer and Probe sets), *T. rubrum* Positive DNA Control and RT Supermix. Furthermore, it contains Buffer A and Buffer B for template preparation. Each of the primer and probe set for pan-dermatophytes and *T. rubrum* detection is designed for a specific region of the *its2* gene, as shown in table 1. The pan-dermatophyte amplification is detected by the fluorescent dye HEX and *T. rubrum* amplification is detected by the fluorescent dye FAM. The Internal Plasmid Control is detected by the fluorescent dye Cy5/Quasar 670. The *T. rubrum* Positive DNA Control contains genomic *T. rubrum* DNA and serves both as control for the pan-dermatophytes and the *T. rubrum* amplification.

Table 1. Target detection of the Primer and Probe Mix

Target detection	Gene
T. rubrum	*its2*
Pan-dermatophytes[1]	*its2*
Internal Plasmid Control	Synthetic fragment

[1](dermatophytes in general e.g. *T. rubrum, T. mentagrophytes, T. interdigitale, T. tonsurans, T. schoeuleinii, T. violaceum, T. soudanense, T. verrucosum, T. erinacei, M. canis, M. audouinii, E. floccosum var. Floccosum*)

Principle

Nail infections are mainly caused by *T. rubrum* and *T. mentagrophytes*. The traditional identification method with culturing and microscopic examination is time-consuming and varies from 10 days to 4 weeks. This multiplex Real Time PCR based method can detect dermatophytes in general, and specifically *T. rubrum* within 2-3 hours.

Materials provided

The reagents supplied in the kit are listed in table 2. The kit comprises reagents enough to perform 100 multiplex Real Time PCR reactions.

Table 2. Reagents provided

Ref	Reagent	Color cap	Volume
1	Primer and Probe Mix [1]	Green	950 µL
2	*T. rubrum* Positive DNA Control	White	150 µL
3	RT Supermix [2]	Red	1,1 mL
A	Buffer A	-	20 mL
B	Buffer B	-	20 mL

[1] Primer and Probe Mix for detection of pan-dermatophytes, *T. rubrum* and Internal Plasmid Control. The plasmid used as template for amplification of internal control is included in the mix as well.
[2] Enzyme master mix for probe-based Real Time PCR.

Instruments required but not provided

- Heat block or water bath (95˚C)
- Vortex mixer
- Real Time Thermal Cycler, which contains FAM, HEX and Cy5/ Quasar 670 channels (see table 3 for wavelengths)
- Computer connected to the Real Time Thermal Cycler for analysis of the results

The Dermatophyte Real Time PCR Kit has been validated on the following instruments: Bio-Rad CFX96™, Qiagen Rotor-Gene® Q and ABI 7500.

Precautions

The Dermatophyte Real Time PCR Kit has been developed for use with template DNA from patient nail specimens prepared using Buffer A and Buffer B (DNA preparation as described below).

If the kit is used with DNA template from cultured fungi, the DNA template must be diluted 100 times with RNase/DNase free water before use.

Procedure

Template preparation and PCR setup should be performed in dedicated areas free of possible contamination.

DNA Preparation

1. Add 100 µL of Buffer A to the nail specimen. Incubate the sample at 95°C for 10 minutes. If the nail specimen is large either cut the nail into small pieces or increase the volume of Buffer A to cover the sample. Increase the volume of Buffer B equally (see next step).
2. Immediately add 100 µL of Buffer B and vortex. The sample is ready for PCR.

PCR Set-up

3. Prepare the master mix as described in table 4 for the number of samples to be run.
4. Dispense 18 µL of the master mix and 2 µL of template DNA (sample or positive control) in each tube. Prepare a negative control (NTC; non-template control) by mixing 18 µL of the master mix and 2 µL Buffer A/Buffer B (ratio 1:1).
5. Set up the PCR protocol on a Real Time Thermal Cycler as described in table 5.
6. Select the fluorescence channel FAM (*T. rubrum*), HEX (pan-dermatophyte) and Cy5/Quasar 670 (Internal Plasmid Control) as described in table 3.

Table 3. PCR target, fluorescent dyes and detection wavelength

Target	Dye	Wavelength (nm)
T. rubrum	FAM	520
Pan-dermatophytes	HEX	556
Internal Plasmid Control	Cy5/Quasar 670	669/670

Table 4. Preparation of the master mix for the PCR Set-up

Reagents	Volume/reaction
Primer and Probe Mix	8 µL
RT Supermix	10 µL
Total volume	18 µL

Table 5. Amplification protocol

Step	Time	Temp.	Cycle(s)
Pre-denaturation	2 min.	95°C	1
Denaturation	10 sec.	95°C	40
Annealing/Extension	1 min.	64°C	

Interpretation of the Analysis Results

In figure 1 the result of a positive *T. rubrum* sample is shown by three amplification curves.

Amplification curve detected by FAM (shown as a **blue** curve): The nail specimen is positive for *T. rubrum* DNA and the patient has a nail infection caused by *T. rubrum*.

Amplification curve detected by HEX (shown as a **green** curve): The nail specimen is positive for pan-dermatophytes and the patient has a nail infection caused by a dermatophyte. In this case the infection is determined to be *T. rubrum*.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple** curve): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative.

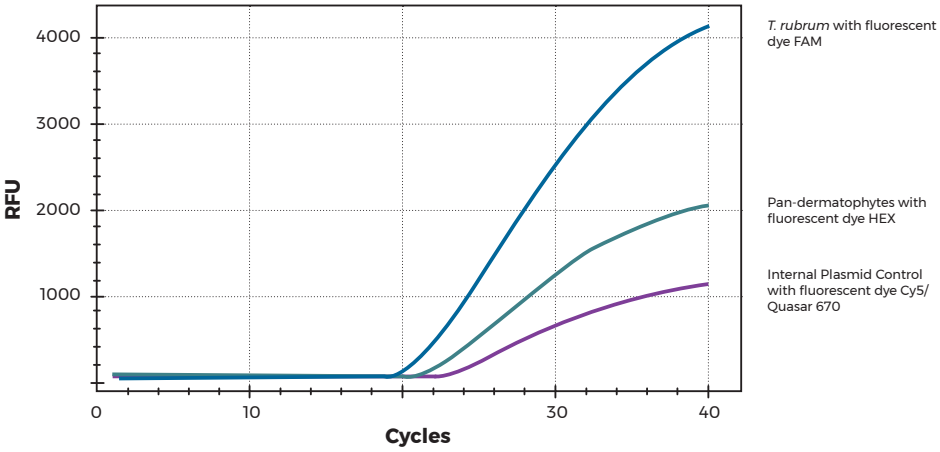

Figure 1: A *T. rubrum* positive nail specimen showing amplification curves for *T. rubrum* (FAM, here shown as **blue**), pan-dermatophytes (HEX, here shown as **green**) and Internal Plasmid Control (Cy5/Quasar 670, here shown as **purple**)

In figure 2 a pan-dermatophyte positive result is shown by two amplification curves.

Amplification curve detected by HEX (shown as a **green** curve): The nail specimen is positive for pan-dermatophytes and the patient has a nail infection caused by a dermatophyte. In this case the nail infection is not caused by *T. rubrum* since an amplification curve detected by FAM is not observed.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple** curve): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative.

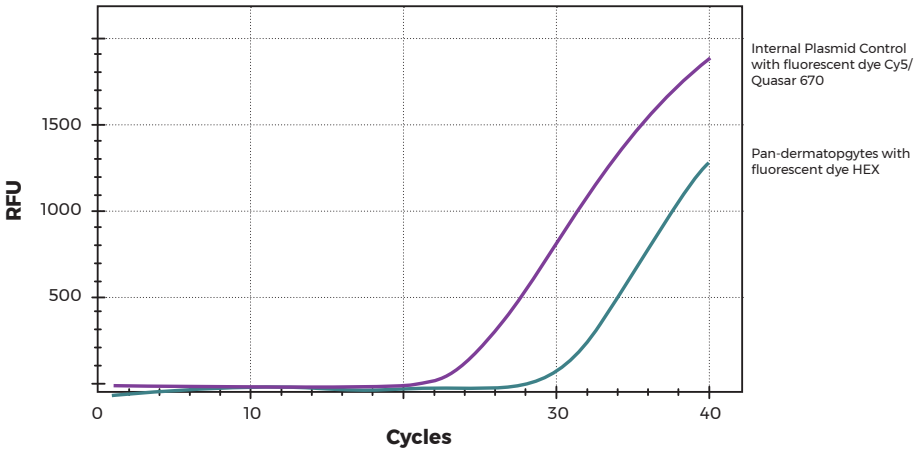

Figure 2: A pan-dermatophytes positive nail specimen shows amplification curve for pan-dermatophytes (HEX, here shown as green) and Internal Plasmid Control fragment (Cy5/Quasar 670, here shown by purple)

In figure 3 the result of a nail sample not infected by a dermatophyte is shown by one amplification curve.

Amplification curve detected by Cy5/Quasar 670 (shown as a **purple** curve): Detection of the Internal Plasmid Control. This signal should be observed in all tests, both positive and negative.

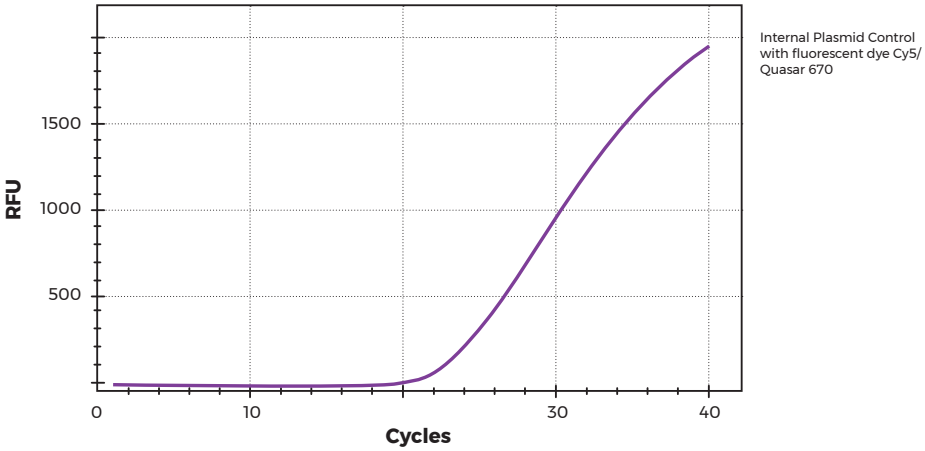

Figure 3: A negative nail specimen shows only an amplification curve for the Internal Plasmid Control fragment (Cy5/Quasar 670, here shown by **purple**)

The amplification curves should start increasing before the Ct values listed in table 6, to be a true positive result.

Table 6. Analysis and interpretation for nail specimens and culture samples

Interpretation	Channel FAM Blue	Channel HEX Green	Channel Cy5/ Quasar 670 Purple
T. rubrum positive	Ct < 37	Ct < 35	Ct < 37
Pan-dermatophytes positive	-	Ct < 35	Ct < 37
Negative	-	-	Ct < 37

Limit of detection
The Dermatophyte Real Time PCR Kit can detect less than 10 copies of genomic DNA of *T. rubrum* and pan-dermatophytes.

Analytical Sensitivity and Specificity
The Dermatophyte Real Time PCR Kit showed an analytical sensitivity of 100% for both *T. rubrum* and dermatophytes detection, when testing a panel of DNA samples purified from 4 *T. rubrum* and 36 other dermatophytes
(*E. floccosum, M. canis, M. audouinii, M. gypseum, T. erinaceid, T. interdigitale, T. mentagrophyte, T. schoeuleinii, T. soudanense, T. tonsurans, T. verrucosum, T. violaceum*).

The Dermatophyte Real Time PCR Kit showed an analytical specificity of 100% when testing a panel of DNA samples purified from 26 non-dermatophytes (*A. flavus, A. fumigatus, A. niger, A. wentii, C. albicans, C. glabrato, C. krusei, C. parapsilosis, C. tropicalis, F. oxysporum, F. proliferatum, F. solani, Mallassezia furfur, S. brevicaulis).*

Accuracy
The Dermatophyte Real Time PCR Kit has been validated with a panel of 187 pan-dermatophyte positive (n= 82) and negative (n= 105) human nail specimens. The accuracy was 98% comparing to results obtained by the hospitals own in-house Real Time PCR test.

Storage and Shelf Life
Store the Dermatophyte Real Time PCR Kit in a dark place at -20˚C.
Avoid repeated thawing/freezing and direct light exposure of the Primer and Probe Mix, as this might reduce the stability of the reagent and thus affect the efficiency of the DNA amplification.

The expiry date of the kit is printed on the label. Do not use the kit after it has expired.

Information and ordering

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